GELS FOR ENCAPSULATION OF BIOLOGICAL MATERIALS

This application is a continuation-in-part of U.S. application Ser. No. 07/870,540, filed on Apr. 20, 1992 now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/843,485, filed on Feb. 28, 1992, now abandoned. This application is also a continuation-in-part of U.S. application Ser. No. 07/598,880, filed Oct. 15, 1990 now abandoned, and U.S. application Ser. No. 07/740,703, filed Aug. 5, 1991 now U.S. Pat. No. 5,380,536, which in turn is a division of U.S. application Ser. No. 07/740,632 filed Aug. 3, 1993 now U.S. Pat. No. 5,232,984. All of the above-identified applications are incorporated herein by reference.

BACKGROUND

Microencapsulation technology holds promise in many areas of medicine. For example, some important applications are treatment of diabetes (Goosen, et al., 1985), production of biologically important chemicals (Omata, et al., 1979), evaluation of anti-human immunodeficiency virus drugs (McMahon, et al., 1990), encapsulation of hemoglobin for red blood cell substitutes, and controlled release of drugs. During encapsulation using prior methods, cells are often exposed to processing conditions which are potentially cytotoxic. These conditions include heat, organic solvents and non-physiological pH which can kill or functionally impair cells. Proteins are often exposed to conditions which are potentially denaturing and can result in loss of biological activity.

Further, even if cells survive processing conditions, the stringent requirements of encapsulating polymers for biocompatibility, chemical stability, immunoprotection and resistance to cellular overgrowth, restrict the applicability of prior art methods. For example, the encapsulating method based on ionic crosslinking of alginate (a polyanion) with polylysine or polyornithine (polycation) (Goosen, et al., 1987) offers relatively mild encapsulating conditions, but the long-term mechanical and chemical stability of such ionically crosslinked polymers remains doubtful. Moreover, these polymers when implanted in vivo, are susceptible to cellular overgrowth (McMahon, et al., 1990) which restricts the permeability of the microcapsule to nutrients, metabolites, and transport proteins from the surroundings. This has been seen to possibly lead to starvation and death of encapsulated islets of Langerhans cells (O'Shea and Sun, 1986).

Thus, there is a need for a relatively mild cell encapsulation method which offers control over properties of the encapsulating polymer. The membranes must be non-toxically produced in the presence of cells, with the qualities of being permselective, chemically stable, and very highly biocompatible. A similar need exists for the encapsulation of biological materials other than cells and tissues.

Biocompatibility

Synthetic or natural materials intended to come in contact with biological fluids or tissues are broadly classified as biomaterials. These biomaterials are considered biocompatible if they produce a minimal or no adverse response in the body. For many uses of biomaterials, it is desirable that the interaction between the physiological environment and the material be minimized. For these uses, the material is considered "biocompatible" if there is minimal cellular growth on its surface subsequent to implantation, minimal inflammatory reaction, and no evidence of anaphylaxis during use. Thus, the material should elicit neither a specific

humoral or cellular immune response nor a nonspecific foreign body response.

Materials which are successful in preventing all of the above responses are relatively rare; biocompatibility is more a matter of degree rather than an absolute state. The first event occurring at the interface of any implant with surrounding biological fluids is protein adsorption (Andrade, et al., 1986). In the case of materials of natural origin, it is conceivable that specific antibodies for that material exist in the repertoire of the immune defense mechanism of the host. In this case a strong immune response can result. Most synthetic materials, however, do not elicit such a reaction. They can either activate the complement cascade or adsorb serum proteins which mediate cell adhesion, called cell adhesion molecules (CAMs) (Buck, et al., 1987). The CAM family includes proteins such as fibronectin, vitronectin, laminin, von Willebrand factor, and thrombospondin.

Proteins can adsorb on almost any type of material. They have positively and/or negatively charged regions, as well as hydrophilic and hydrophobic regions. They can thus interact with implanted material through any of these various regions, resulting in cellular proliferation at the implant surface. Complement fragments such as C3b can be immobilized on the implant surface and act as chemoattractants. They in turn can activate inflammatory cells such as macrophages and neutrophils and cause their adherence and activation on the implant. These cells attempt to degrade and digest the foreign material.

In the event that the implant is nondegradable and is too large to be ingested by large single activated macrophages, the inflammatory cells may undergo frustrated phagocytosis. Several such cells can combine to form foreign body giant cells. In this process, these cells release peroxides, hydrolytic enzymes, and chemoattractant and anaphylactic agents such as interleukins, which increase the severity of the reaction. They also induce the proliferation of fibroblasts on foreign surfaces.

Fibroblasts secrete a collagenous matrix which ultimately results in encasement of the entire implant in a fibrous envelope. Cell adhesion can also be mediated on a charged surface by the cell surface proteoglycans such as heparin sulfate and chondroitin sulfate (van Wachem, et al., 1987). In such a process, intermediary CAMs are not required and the cell surface can interact directly with the surface of the implant.

Enhancing Biocompatibility

Past approaches to enhancing biocompatibility of materials started with attempts at minimization of interfacial energy between the material and its aqueous surroundings. Similar interfacial tensions of the solid and liquid were expected to minimize the driving force for protein adsorption and this was expected to lead to reduced cell adhesion and thrombogenicity of the surface. For example, Amudeshwari et al. used collagen gels cross-linked in the presence of HEMA and MMA (Amudeshwari, et al., 1986). Desai and Hubbell showed a poly(HEMA)-MMA copolymer to be somewhat non-thrombogenic (Desai, N. P. and Hubbell, 1989).

Protein adsorption and desorption, however, is a dynamic phenomenon, as seen in the Vroman effect. This effect is the gradual displacement of one serum protein by another, through a well-defined series, until only virtually irreversibly adsorbed proteins are present on the surface. Affinity of protein in a partially dehydrated state for the polymer surface has been proposed as a determining factor for protein adsorption onto a surface (Baier, 1990). Enhancement of surface hydrophilicity has resulted in mixed suc-